Analysis of Uncertainties in Dose Reconstruction from Biomarkers: Impact on Study Design

Miles S. Okino[1], James J. Quackenboss[1], and Kent W. Thomas[2]
[1]US EPA/Human Exposure Research Branch, PO Box 93478, Las Vegas, NV 89193-3478, [2]US EPA/Human Exposure Analysis Branch, Research Triangle Park, NC 27711

1. Abstract

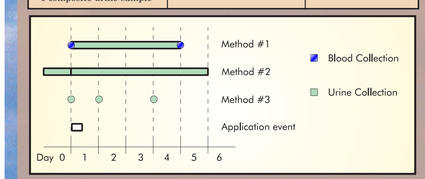
The absorbed dose of a pesticide can be estimated from its established urinary biomarker. Absorbed dose is defined as the quantity which has passed through the exchange boundaries (skin, GI tract, lungs). For a study focused on application events, there are several options for biomarker collection, each with advantages and disadvantages in terms of model and scenario uncertainty, subject participation and compliance, and cost. Model uncertainty includes the parameters used for the calculation of dose as well as the ability of the model to simulate the real physical situation. Scenario uncertainty includes the activities of the subject such as other exposures to the target chemical, compliance with measurement protocols, and the timing of the monitored dose. We will demonstrate the effect of scenario and model uncertainties on the dose estimates under various design options, and how the uncertainty analysis may be used to guide the design of a study. For an evaluation of 2,4-D applicators, we will compare the uncertainty associated with collecting spot samples versus total urine collection.

2. Introduction

- Biomarkers can be used to estimate the quantity of absorbed dose from a pesticide application event
- Proper timing of biomarker collection is important to minimize the range of uncertainty
  - *Model uncertainty* includes parameters used to calculate dose as well as the ability of a model to simulate the actual dynamics of the chemical in the body
  - *Scenario uncertainty* is a result of not knowing the exact time profile of exposure, other exposures that may occur other than the monitored application event, and non-compliance with the measurement protocols
- The optimum collection scheme balances the uncertainty range against the collection burden and study costs
  - Increased rates of subject participation and compliance with measurement protocols
  - Ability to include more subjects in a study

3. Biomarker Collection Options for Absorbed Dose Estimation from an Application Event

Total Urine with Blood Collection Method #1	Total Urine Collection Method #2	Spot Urine Collection Method #3
Collect total urine for a designated number of days beginning with the exposure event, and blood measurements at the beginning and end of the study period	Collect total urine for 24 hours before, and sufficient days after the application event to ensure that all the absorbed chemical has been excreted	Collect spot urine (morning void) samples before the application event, 1 day after, and 3 days after the application event
2 Blood samples 1 composite urine sample	2 urine samples	3 urine samples



4. Comparison of Collection Options

Total Urine with Blood Collection Method #1	Total Urine Collection Method #2	Spot Urine Collection Method #3
High burden placed on the applicator		Simplest collection protocol
Assume that no other exposure occurs after the monitored event		Able to incorporate information from time-activity data
Assume that blood/body partitioning is known	Assume that the fraction of the absorbed dose excreted by the end of the collection period is known from the half-life	Assume a reliable pharmacokinetic (PK) model exists for the chemical and that the parameter values are relevant to the population of interest
	Assume that only the background exposure (steady state) has occurred during the period starting 5-8 days prior to the application day	Exposure profiles modeled from time-activity data
Introduces the least model uncertainty		Highest model uncertainty introduced

5.

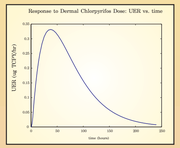
Total urine with blood (Method #1)

Absorbed dose=(total final body content+mass exceted in urine)-total initial body content

Total urine collection (Method #2)

- The length of the collection period is determined by the rate at which the metabolite is excreted into the urine: urinary excretion rate, UER [μg/hr]
- The objective is to collect the total mass attributable to the application event
- This total mass is represented by the Area Under the Curve (AUC), which is the integral of the urinary excretion rate (UER) over time from 0 to ∞

UER Response to a dermal chlorpyrifos dose

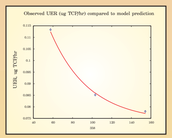


- Assuming that no significant exposure occurred for a period before or after the monitored event, and the collection period is sufficiently long to capture all the metabolite:

mass excreted in urine=mass absorbed during application event

6. Estimate a dose based on a series of spot urine measurements (Method #3)

Model (solid line) fit to UER values ( ) calculated from biomarker measurements



The UER calculated from the biomarker measurements and an inverted PK model are used to calculate a dose [μg/kg body weight (bw)]

Urine Concentration (C_u [μg/L]) ⇨ Average Urinary Excretion Rate ⇨ Absorbed Dose

and void volume (V_u [L]) $\quad \overline{UER} = \frac{C_u V_u}{t_{previous\ void}} \quad dose\left[\frac{\mu g}{kg\ bw}\right] = f(C_u, V_u, \mathbf{t}, \mathbf{k})$

where **t** is the vector of relevant times (time since exposure and previous void) and **k** is the vector of PK model parameters

- The use of a dynamic model enables the incorporation of information about other exposures:
  - Other (not monitored) application events reported on questionnaires
  - High residential concentrations
- Contributions from other routes and time periods are assumed to be additive (linear model)

7. Model Uncertainties Chlorpyrifos PK Model Example

Multiplicative Factors

Selectivity[1,2]	0.53-1.0	fraction of absorbed chlorpyrifos that is excreted as urinary TCPY
TCP concentration[1,2]	+/- 15%	measurement error
Oral fraction absorbed	0.53-0.97	based on Nolan et al., 1984
Dermal fraction absorbed	0.003-0.075	
Recorded sample size[1,2]	+/- 5%	measurement error (volume reading)

Time Dependent Factors

Elimination rate constant[2] [hr^{-1}]	0.018-0.036	based on Nolan et al., 1984
Oral absorption rate constant [hr^{-1}]	0.075-10.8	
Dermal absorption rate constant [hr^{-1}]	0.018-0.50	
Recorded duration of time since last void	+/- 1 hour	subject's recollection

[1]Parameters also used to estimate dose in total urine collection schemes (Methods #1 and #2)

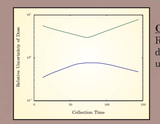
[2]Parameters also used to estimate dose in the total urine collection scheme without blood measurements (Method #2)

Nolan, R.J.; Rick, D.L.; Freshour, N.L.; Saunders, J.H. Chlorpyrifos: Pharmacokinetics in Human Volunteers, *Toxicol. Appl. Pharm.* 1984, 73, 8-15.

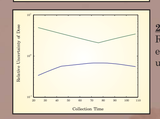
8.

Model Uncertainty vs. Time

Profiles were constructed from a numerical sensitivity analysis of the associated inverse PK models for determination of optimum sample collection time points



Chlorpyrifos Example
Relative uncertainty in absorbed dermal chlorpyrifos dose estimate vs. collection time. The minimum relative uncertainty occurs at a time of 72 hours.



2,4-D Acid Example
Relative uncertainty in absorbed dermal 2,4-D acid dose estimate vs. collection time. The minimum relative uncertainty occurs at a time of 76 hours.

Conclusion
For these compounds, suitable collection times would be the first morning voids on the first day (24 hours, close to maximum UER) and third day (72 hours, uncertainty minimum) after the application event

9. Model Uncertainty Comparison: Chlorpyrifos Example

Ranges in Total Absorbed Oral Dose (μg/kg body weight) Estimations Reflecting the Model Uncertainty

Total Absorbed Dose of Chlorpyrifos	Spot Urine when sample volume and time *not* recorded	Method #3: Spot Urine with sample volume and time recorded	Method #2: Ideal[1] Total Urine (5 day collection)	Method #1: Ideal[1] Total Urine with Blood Measurements
μg/kg body weight	1.4-13.6	2.0-10.2	2.6-8.9	2.6-8.0

[1]Ideal total urine schemes assume that no exposure to chlorpyrifos occurred after the studied event and that the blood/body partitioning is known exactly.

Note that the ideal total urine (Method #2) uncertainty range will approach the urine with blood (Method #3) uncertainty as the collection period is extended past 5 days.

Conclusion
In terms of the model uncertainty, it is possible to estimate dose with a similar range of uncertainty using spot urine samples rather than total urine collection schemes.

10. Comparison to Field Data (Nash et al., 1982) Spot Urine vs. Total Urine Collection: 2,4-D Amine Salt Application

7 Days of total urine was collected, partitioned into 24 hour samples (1 day before application, 6 days following)

Collection period was too short to estimate dose by a total mass balance (Method #2)

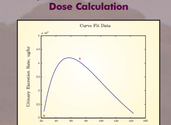
- Applying Method #3 (spot urine samples), we used only two readings to estimate dose: First and third days after application (24 and 72 hours after application)
- Model and scenario uncertainty are important
- Simulate the model using the calculated dose (Method #3) and compare to the full set of observations
- Compare the model predicted mass excreted and observed mass excreted

Nash, R.G.; Kearney, P.C.; Maillen, J.C.; Sell, C.R.; Fertig, S.N. "Agricultural applicators exposure to 2,4-dichlorophenoxyacetic acid," in *Pesticide Residues and Exposure*, J.R. Plimmer (Ed.), ACS Symp. Ser. No. 182, 1982, p. 119-132.

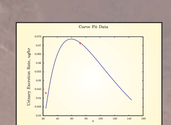
11.

Model Predicted UER (solid line) and Data Points used in Dose Calculation

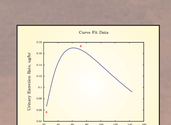
Model Predicted UER (solid line) and All Data Points



Inverse PK model fit to the observed UER for subject 1.



Inverse PK model fit to the observed UER for subject 6.



Inverse PK model fit to the observed UER for subject 22.

12. Comparison of Observed and Model Predicted Excreted Metabolite Mass and Absorbed Dose. Observed data from Nash et al., 1982.

Subject	Observed Excreted Mass of 2,4-D Acid (6 days)	Calculated (Method #3) Excreted Mass of 2,4-D Acid (6 days)	Calculated (Method #3) Absorbed Dose (acid equivalent) of 2,4-D Amine Salt
	μg/kg body weight	μg/kg body weight	μg (a.e.)/kg body weight
1	0.30	0.45 [0.28 - 3.2]	0.62 [0.38 - 4.4]
6	5.95	7.8 [4.8 - 55]	11 [6.7 - 77]
22	20.1	18 [11 - 130]	25 [15 - 180]

- Scenario uncertainty was a major factor in the differences in excreted 2,4-D based on the full set of data points compared to the inverse PK model prediction (Method #3)
- The wide range of dose estimates are due to large uncertainties in the PK model absorption parameters
- Note that the 6 day collection period appears to be too short to apply an overall mass balance to calculate absorbed dose (Method #2)

13. Conclusions

- Possible to estimate dose with similar uncertainty ranges using spot urine samples in place of total urine collection schemes
- Analysis of the model uncertainty can direct the design of a biomarker collection scheme that will provide reliable dose estimates at minimal cost and subject burden
- Narrowing scenario uncertainty is important for accurate estimation of dose from biomarkers

Future Research Needs

- Methods will be applicable for a wider range of chemicals as PK models are developed
- Reduction of model uncertainty will be possible as relevant data sets for the estimation of PK model parameters are obtained
- Reduced scenario uncertainty is dependent on improved analysis and classification of environmental concentration measurements, questionnaires, and activity data